

In the Specification

Please insert the following paragraph before the first paragraph on page 1 of the specification, above [Field of industrial application].

Page 1, paragraph 1 (NEW)

This application is a divisional of U.S. Patent Application Serial No. 09/582,926, now U.S. Patent No. \_\_\_\_\_, issued \_\_\_\_\_, which was the National Phase filing of International Patent Application No. PCT/JP99/00086, filed January 13, 1999.

Please substitute the following paragraph for the first paragraph on page 1 of the specification.

Page 1, paragraph 1 (Currently Amended)

The present invention relates to a sustained-release composition of a biologically active substance, **and** a production method thereof.

Please substitute the following paragraph for the fifth paragraph on page 1 of the specification.

Page 1, paragraph 5 (Currently Amended)

To provide a novel composition that contains a **high concentration of** biologically active substance ~~at high contents~~, and that is capable of ~~controlling the rate of its~~ **a controlled rate of** release.

Please substitute the following paragraph for the sixth paragraph starting on page 1 of the specification.

Page 1, paragraph 6 (Currently Amended)

After extensive investigation aiming at resolving the above problem, the present inventors found that when ~~the a~~ a biologically active substance is incorporated ~~at high contents in~~ high concentration in ~~the a~~ a composition by allowing the biologically active substance and the hydroxynaphthoic acid to be co-present during formation of the composition, and when both are included in ~~the a~~ a biodegradable polymer, the biologically active substance is released at rates differing from those of the biologically active substance from the counterpart composition of the biologically active substance and hydroxynaphthoic acid prepared in the absence of the biodegradable polymer, which rate of release ~~being is~~ is controllable by choosing the appropriate kind of biodegradable polymer. The inventors conducted further ~~investigation~~ investigations based on this finding, and developed the present invention.

Please substitute the following paragraph for the fifth paragraph on page 3 of the specification.

Page 3, paragraph 5 (Currently Amended)

(11) a sustained-release composition according to term (3) above, wherein the LH-RH derivative is a peptide represented by the formula:

5-oxo-Pro-His-Trp-Ser-Tyr-Y-Leu-Arg-Pro-Z (SEQ ID NO.:1)

wherein Y represents DLeu, DAla, DTrp, DSer(tBu), D2Nal or DHis(ImBzl); Z represents NH-C<sub>2</sub>H<sub>5</sub> or Gly-NH<sub>2</sub>,

Please substitute the following paragraph for the sixth paragraph on page 4 of the specification.

Page 4, paragraph 6 (Currently Amended)

(22) an agent for preventing or treating ~~of~~ prostatic cancer, prostatic hypertrophy, endometriosis, hysteromyoma, metrofibroma, precocious puberty, dysmenorrhea, or breast cancer, or a contraceptive, containing the sustained-release composition according to term (3) above,

Please substitute the following paragraph for the first paragraph on page 6 of the specification.

Page 6, paragraph 1 (Currently Amended)

~~Although the~~ The biologically active substance used in the present invention is not subject to limitation, as long as it is pharmacologically useful, and it may be a non-peptide substance or a peptide substance. The non-peptide substance includes an agonist, an antagonist, and a substance having an enzyme inhibitory activity. The peptide substance includes, for example, ~~a~~ biologically active peptides, and particularly those having molecular weights of about 300 to about 40,000, preferably about 400 to about 30,000, and more preferably about 500 to about 20,000 ~~are preferred~~.

Please substitute the following paragraph for the fourth paragraph starting on page 6 of the specification.

Page 6, paragraph 4 (Currently Amended)

Such salts include salts with inorganic acids (~~it~~ which may also be ~~called~~ referred to as inorganic free acids) (e.g., carbonic acid, bicarbonic acid, hydrochloric acid, sulfuric acid, nitric acid, boric acid), organic acids (~~it~~ which may also be ~~called~~ referred to as organic free acids)(e.g., succinic acid, acetic acid, propionic acid, trifluoroacetic acid) etc., when said biologically active peptide has a basic group such as an amino group.

Please substitute the following paragraph for the second paragraph on page 7 of the specification.

Page 7, paragraph 2 (Currently Amended)

When said biologically active peptide has an acidic group such as a carboxyl group, such salts include salts with inorganic bases (~~it~~ which may also be ~~called~~ referred to as inorganic free bases) (e.g., alkali metals such as sodium and potassium, alkaline earth metals such as calcium and magnesium), organic bases (~~it~~ which may also be ~~called~~ referred to as organic free bases) (e.g., organic amines such as triethylamine, basic amino acids such as arginine) etc. The biologically active peptide may form a metal complex compound (e.g., copper complex, zinc complex).

Please substitute the following paragraph for the fifth paragraph starting on page 7 of the specification.

Page 7, paragraph 5 (Currently Amended)

LH-RH derivatives may be LH-RH agonists or LH-RH antagonists; useful LH-RH antagonists include, for example, biologically active peptides represented by general formula [I]:  
X-D2Nal-D4ClPhe-D3Pal-Ser-A-B-Leu-C-Pro-DAlaNH<sub>2</sub> (**SEQ ID NO.: 2**) [X represents N(4H<sub>2</sub>-furoyl)Gly or NAc; A represents a residue selected from NMeTyr, Tyr, Aph(Atz) and NMeAph(Atz); B represents a residue selected from DLys(Nic), DCit, DLys(AzaglyNic), DLys(AzaglyFur), DhArg(Et<sub>2</sub>), DAph(Atz) and DhCi; C represents Lys(Nisp), Arg or hArg(Et<sub>2</sub>)] or salts thereof.

Please substitute the following paragraph for the second paragraph on page 8 of the specification.

Page 8, paragraph 2 (Currently Amended)

Useful LH-RH agonists include, for example, biologically active peptides represented by general formula [II]:

5-oxo-Pro-His-Trp-Ser-Tyr-Y-Leu-Arg-Pro-Z (**SEQ ID NO.: 1**)

[Y represents a residue selected from DLeu, DAla, DTrp, DSer(tBu), D2Nal and DHis(lmBzl); Z represents NH<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>, Gly-NH<sub>2</sub>] or salts thereof. Peptides wherein Y is DLeu and Z is NH-C<sub>2</sub>H<sub>5</sub>, (i.e., a peptide represented by the formula: 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> (**SEQ ID NO.: 3**)) in particular, are preferred.

Please substitute the following paragraph for the second paragraph on page 12 of the specification.

Page 12, paragraph 2 (Currently Amended)

The mode of monomer binding may be random, block, or graft. When the above-mentioned  $\alpha$ -hydroxymonocarboxylic acids,  $\alpha$ -hydroxydicarboxylic acids, and  $\alpha$ -hydroxytricarboxylic acids have an ~~optical~~ optically active center in their molecular structures, they may be of the D-, L- or DL-configuration. Of these, lactic acid-glycolic acid polymers [hereinafter also referred to as poly(lactide-co-glycolide), poly(lactic acid-co-glycolic acid) or lactic acid-glycolic acid copolymer; generically refer to lactic acid-glycolic acid homopolymers and copolymers, unless otherwise specified; lactic acid homopolymers are also referred to as lactic acid polymer, polylactic acids, polylactides etc., and glycolic acid homopolymers as glycolic acid polymers, polyglycolic acids, polyglycolides etc.], with preference given to poly( $\alpha$ -cyanoacrylic esters) etc. Greater preference is given to lactic acid-glycolic acid polymers. More preferably, lactic acid-glycolic acid polymers having a free carboxyl group at one end are used.

Please substitute the following paragraph for the second paragraph on page 19 of the specification.

Page 19, paragraph 2 (Currently Amended)

The method of polymerization of the present application employs hydroxycarboxylic acid derivatives (e.g., tert-butyl D-lactate, benzyl L-lactate) with a protected carboxyl group or hydroxydicarboxylic acid derivatives (e.g., dibenzyl tartronate, di-tert-~~butyl~~ butyl L-2-hydroxyethylmalonate) with a protected carboxyl, in place of conventional protonic chain transferring agents such as methanol.

Please substitute the following paragraph for the third paragraph on page 33 of the specification.

Page 33, paragraph 3 (Currently Amended)

First, an organic solvent solution containing a hydroxynaphthoic acid and a biodegradable polymer is prepared. Thus-obtained organic solvent solution is ~~called~~ **referred to** as an oil phase. The preparation method is the same as those described in paragraph (I) (i) above.

Please substitute the following paragraph for the fourth paragraph on page 33 of the specification.

Page 33, paragraph 4 (Currently Amended)

~~Alternatively~~ **Alternatively**, an organic solvent solution containing a hydroxynaphthoic acid and an organic solvent solution containing a biodegradable polymer may be prepared separately, and mixed together to prepare the oil phase.

Please substitute the following paragraph for the first paragraph on page 34 of the specification.

Page 34, paragraph 1 (Currently Amended)

Next, a solution of a biologically active substance or salt thereof [this solvent exemplified by water, alcohols (e.g., methanol, ethanol)] is prepared. Thus-obtained solution is ~~called~~ **referred to** as internal water phase. The concentration of the biologically active substance is normally 0.001mg/ml to 10g/ml, preferably, 0.1mg/ml to 5g/ml, more preferably, 10mg/ml to 3g/ml. The oil phase and the internal water phase are emulsified by a known method such as

homogenization or sonication to form a W/O emulsion.

Please substitute the following paragraph for the fourth paragraph on page 34 of the specification.

Page 34, paragraph 4 (Currently Amended)

Thus-obtained W/O ~~w/o~~ emulsion containing a biologically active substance or salt thereof, hydroxynaphthoic acid or salt thereof, and a biodegradable polymer, is then added to a water phase to form a w(internal water phase)/o(oil phase)/w(external water phase) emulsion, after which the solvent is evaporated from the oil phase to ~~yeild~~ yield microspheres. For this operation, the external water phase volume is normally chosen over the range from about 1 time to about 10,000 times, preferably from about 2 times to about 100 times, and more preferably from about 3 times to about 10 times, the internal water phase volume.

Please substitute the following paragraph for the second paragraph on page 37 of the specification.

Page 37, paragraph 2 (Currently Amended)

Said organic solvent is the same as those described in ~~paregraph~~ paragraph (I) (i) above. When ~~none~~ more than two kinds of organic solvents are used as a mixed solvent, the ratio of mixture is the same as those described in paragraph (I) (i) above.



Please substitute the following paragraph for the second paragraph on page 42 of the specification.

Page 42, paragraph 2 (Currently Amended)

The sustained-release composition of the present invention is useful, depending on the biologically active substance which is contained in the sustained-release composition, as an agent for treating or preventing various kinds of diseases. When the biologically active substance is LH-RH derivatives, the sustained-release composition of the present invention is useful as an agent for treating or preventing of hormone-dependent diseases, especially sex hormone-dependent diseases, such as sex hormone-dependent cancer (e.g. prostatic cancer, hystero carcinoma, breast cancer, hypophysoma, etc.), prostatic hypertrophy, endometriosis, hystero myoma, precocious puberty, dysmenorrhea, amenorrhea, premenstrual syndrome, multilocular-ovary syndrome. The sustained-release composition of the present invention is also useful for an ~~anticonceptice~~ anticonceptive agent. When the ~~re-bound~~ rebound effect of after medication is used, the sustained-release composition of the present invention is useful as an agent for treating or preventing of ~~infreundity~~ infecundity. Further, the sustained-release composition of the present invention is useful as an agent for treating or preventing of sex hormone-nondependent, but LH-RH sensitive benign or cacoethic neoplasm.

Please substitute the following paragraph for the fourth paragraph on page 42 of the specification.

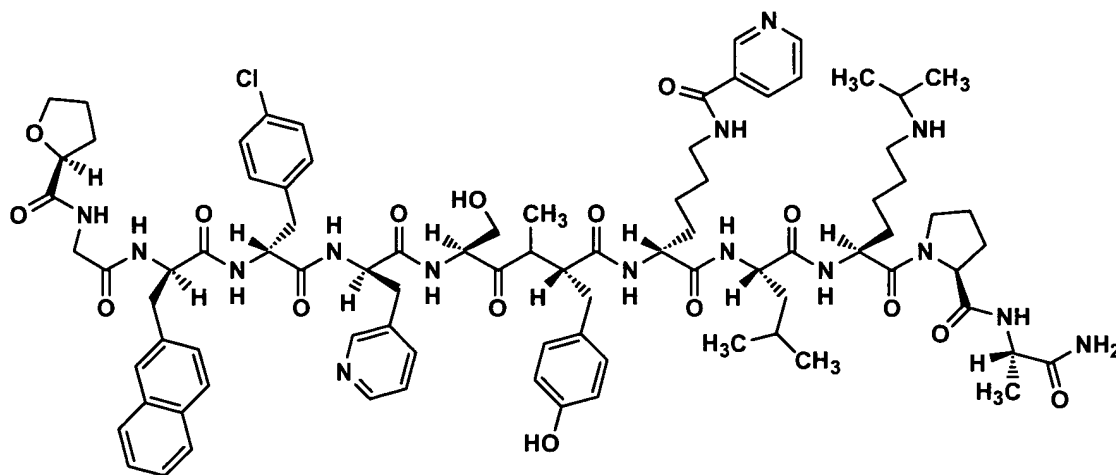
Page 42, paragraph 4 (Currently Amended)

3,429.6 mg of the acetate (produced by TAP) of N-(S)-tetrahydrofur-2-oyl-Gly-D2Nal-D4ClPhe-D3Pal-Ser-NMeTyr-DLys(Nic)-Leu-Lys(Nisp)-Pro-DAlaNH<sub>2</sub> (hereinafter referred to

as peptide A (SEQ ID NO.: 4)

(Chemical Formula of peptide A)

Please replace the structure at the top of page 43 of the specification with the following structure.



Please substitute the following paragraph for the second paragraph starting on page 47 of the specification.

Page 47, paragraph 2 (Currently Amended)

A solution of 0.8 g of the acetate of 5-oxo-Pro-His-Trp-Ser-Tyr-DLeu-Leu-Arg-Pro-NH-C<sub>2</sub>H<sub>5</sub> (hereinafter referred to as peptide B, produced by Takeda Chemical (SEQ ID NO.: 5)) in 0.8 ml of distilled water was mixed with a solution of 3.08 g of a DL-lactic acid polymer (weight-average molecular weight 36,000, number-average molecular weight 18,000, carboxyl group content based on labeling quantitation method 70.4  $\mu$ mol/g) and 0.12 g of 3-hydroxy-2-

naphthoic acid in a mixed organic solvent of 5 ml of dichloromethane and 0.3 ml of ethanol, and this mixture was emulsified in a homogenizer to yield a W/O emulsion. This W/O emulsion was injected to 800 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry), previously adjusted to 15°C, and stirred at 7,000 rpm using a turbine type homomixer to yield a W/O/W emulsion. This W/O/W emulsion was stirred at room temperature for 3 hours to volatilize or diffuse in the external aqueous phase the dichloromethane and ethanol, to solidify the oil phase, after which the oil phase was sieved through a sieve of 75 µm pore size, followed by centrifugation at 2,000 rpm for 5 minutes in a centrifuge (05PR-22, Hitachi, Ltd.) to sediment microcapsules, which were collected. The microcapsules were again dispersed in distilled water, then centrifuged, followed by washing free drug etc. and microcapsule collection. The microcapsules were re-dispersed in a small amount of distilled water added, after which they were freeze-dried to yield a powder. The microcapsule mass recovery rate was 46%, the microcapsule peptide B content and 3-hydroxy-2-naphthoic acid content being 21.3% and 2.96%, respectively. The inclusion efficiencies as determined by dividing these actual contents by the respective charge contents were 106.6% for peptide B and 98.6% for 3-hydroxy-2-naphthoic acid.

Please substitute the following paragraph for the second paragraph starting on page 50 of the specification.

Page 50, paragraph 2 (Currently Amended)

A solution of 1.2 g of the acetate of peptide B in 1.2 ml of distilled water was mixed with a solution of 4.8 g of the same DL-lactic acid polymer as of Example 9 in 7.8 ml of dichloromethane, and this mixture was injected to 1,200 ml of a 0.1% (w/w) aqueous solution of

polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry), previously adjusted to 15°C, and stirred at 7,000 rpm using a turbine type homomixer to yield a W/O/W emulsion. This W/O/W emulsion was treated in the same manner as in Example 8 to yield a microcapsule powder. The microcapsule mass recovery rate as determined by subtracting the amount of mannitol added was 53.6%, the microcapsule peptide B content being 12.1%. The peptide B inclusion efficiency as determined by dividing ~~these~~ the actual content by the charge content was 60.6%, a rate much lower than that obtained in Example 9. It is therefore evident that peptide B inclusion efficiency was increased by the addition of 3-hydroxy-2-naphthoic acid.

Please substitute the following paragraph for the first paragraph on page 56 of the specification.

Page 56, paragraph 1 (Currently Amended)

As seen in Tables 2 and 3, the microcapsules described in Examples 7 through 12 show dramatically higher retention rates of about 90% or higher at 1 day after administration, as compared with Comparative Example 1. It is therefore evident that 3-hydroxy-2-naphthoic acid is effective not only in allowing bioactive substance incorporation at high contents in sustained-release preparations, but also in very well suppressing initial burst of bioactive substances. Experiments using the microcapsules described in Examples 7 through 9, in particular, demonstrate that when DL-lactic acids with weight-average molecular weights of about 20,000 to about 50,000, and carboxyl group contents ~~f~~ of about 50 to 90  $\mu\text{mol/g}$ , as determined by the labeling quantitation method, are used, it is possible to release a bioactive substance at a constant rate over a very long period of time.

Please substitute the following paragraph for the third paragraph starting on page 58 of the specification.

Page 58, paragraph 3 (Currently Amended)

The sustained-release composition of the present invention contains a biologically active substance at high ~~contents~~ concentration, and is capable of ~~controlling the rate of it's a~~ controlled rate of release.